Oomycetes seek help from the plant

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Spotlight

Oomycetes Seek Help from the Plant: *Phytophthora infestans* Effectors Target Host Susceptibility Factors

Plants have a sophisticated immune system to defend against a wide range of invaders, including insects, nematodes, bacteria, oomycetes, fungi, and viruses. Microbes may manipulate or suppress immunity by delivering effector proteins, either to the inside or outside of plant cells. Much attention has been focused on identifying the targets of effector proteins in the host and on characterizing how effector activities suppress immunity. The best studied effector proteins are bacterial type III effectors (T3Es), many of which target positive regulators of immunity in order to inhibit their activity (Deslandes and Rivas, 2012). Yet some bacterial T3E effectors have been found to target host proteins that either negatively regulate immunity, or directly benefit bacterial nutrition. Examples include the *Psuedomonas syringae* T3E AvrB, which mediates phosphorylation and consequent activation of MPK4, a suppressor of immunity (Cui et al., 2010); and the *Xanthomonas* Transcriptional Activator-like effectors which directly upregulate *SWEET* genes encoding the proteins involved in sugar efflux and thus providing pathogen nutrition (Chen et al., 2010). The targets of these effectors are so-called susceptibility (S) factors, proteins whose activity supports a compatible interaction. S factors can facilitate pathogen penetration, negatively regulate immunity, or fulfill metabolic or structural needs of the pathogen during infection. Their mutation often compromises disease development (van Schie and Takken, 2014).

Phytophthora infestans is an oomycete and causal agent of late blight, the major disease of potato. Like other oomycetes, *P. infestans* delivers so-called RXLR effectors into plant cells to suppress immunity (Anderson et al., 2015). As is the case with bacterial T3Es, *P. infestans* RXLRs have been shown to target host proteins and prevent their activity or normal function. An example is the effector RD2 which targets MAP3Kε, a positive regulator of cell death triggered by the tomato receptor Cf4. RD2 directly inhibits MAP3Kε activity (King et al., 2014). In this Spotlight, we highlight three recently described *P. infestans* RXLR effectors, all of which surprisingly target host proteins whose activity enhances susceptibility.

P. INFESTANS RXLR EFFECTORS TARGET SUSCEPTIBILITY FACTORS

The RXLR effector Pi04089 interacts with a K-homology (KH) RNA-binding protein, KRBP1, at speckles in the nucleus. KRBP1 protein abundance is increased when it is co-expressed with Pi04089. It is also increased during the first 24 h after *P. infestans* inoculation, an early stage of the biotrophic phase of infection. Remarkably, transient overexpression of *KRBP1* enhances *P. infestans* leaf colonization, revealing that it promotes susceptibility. Mutation of the KH domains of KRBP1, preventing interaction with RNA, leads to a non-functional form that fails to enhance *P. infestans* colonization (Wang et al., 2015). Thus, KRBP1 can be regarded as an S factor, as its activity is beneficial to the pathogen.

P. infestans RXLR effector Pi04314/RD24 interacts with three host isoforms of protein phosphatase PP1 catalytic subunits, causing their re-localization from the nucleolus to the nucleoplasm. PP1c re-localization from the nucleolus is also observed during infection in host cells that are in intimate contact with *P. infestans* haustoria. Interaction and re-localization are dependent on an R/KVxF motif in Pi04314, indicating that the effector mimics regulatory subunits of PP1c. Remarkably, virus-induced gene silencing (VIGS) of interacting PP1c subunits significantly reduces *P. infestans* leaf colonization, indicating that the pathogen requires host PP1c protein, and/or its activity, for late blight infection to develop. Indeed, Pi04314 does not inhibit PP1c phosphatase activity. Transient expression of a mutant form of PP1c in which phosphatase activity is abolished results in reduced pathogen colonization (Boevink et al., 2016). It is thus likely that Pi04314 forms novel holoenzymes with selected PP1c isoforms, directing their activity to the nucleoplasm to dephosphorylate key substrates. Transgenic plants expressing Pi04314 are attenuated in salicylic acid and jasmonic acid transcriptional responses, suggesting that Pi04314-PP1c holoenzymes negatively regulate these defense pathways (Boevink et al., 2016).

The third *P. infestans* effector of interest in this context is Pi02860. This effector suppresses cell death triggered by the *P. infestans* pathogen-associated molecular pattern (PAMP) INF1 (Yang et al., 2016). Unlike RD2, referred to above (King et al., 2014), Pi02860 does not suppress Cf4-triggered cell death. Pi02860 interacts with a host non-phototrophic hypocotyl 3/root phototropism 2 (NPH3/RPT2)-like protein, NRL1, in the cytoplasm and at the cell plasma membrane. NRL1 is a Broad-Complex, Tramtrack and Bric-a-brac (BTB/POZ) domain protein and, as such, is a predicted Cullin 3-associated ubiquitin E3 ligase. Remarkably, transient overexpression of *NRL1* results in suppression of INF1-triggered cell death and enhances *P. infestans* leaf colonization. Conversely, silencing of *NRL1* by VIGS accelerates INF1-triggered cell death and reduces pathogen colonization. Moreover, VIGS of *NRL1* specifically attenuates the ability of Pi02860 to attenuate INF1-triggered cell death (Yang et al., 2016). NRL1 is a negative regulator of immunity, and thus an S factor targeted by a *P. infestans* RXLR effector.

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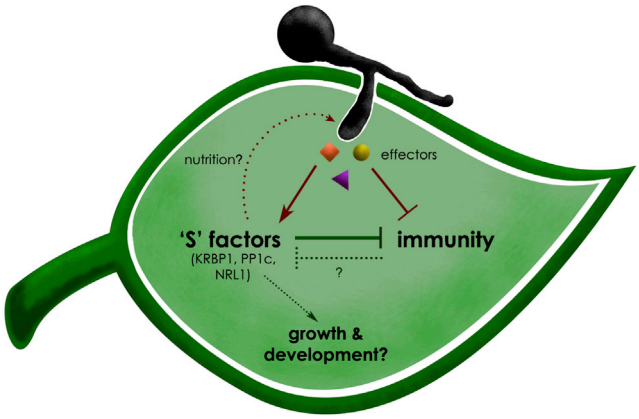

Figure 1. *Phytophthora infestans* Effectors May Target Susceptibility Factors to Antagonize Immunity.
Schematic of an infected leaf cell showing that the oomycete *Phytophthora infestans* delivers RXLR effector proteins from haustoria to the inside of host cells where they may directly target and inhibit positive regulators of immunity. In addition, effectors may target host proteins that act as susceptibility (S) factors (PP1c, KRBP1, NRL1), supporting or promoting their activity. Susceptibility factors can negatively regulate immunity. Future work will reveal whether S factors also positively regulate growth and development in the plant, and/or whether they enhance pathogen nutrition. Moreover, as S factors such as NRL1 act as negative regulators of immunity in the absence of the *P. infestans* effector, it is anticipated that NRL1 will itself be negatively regulated during PAMP-triggered immunity.

CONCLUSIONS AND FUTURE DIRECTION

The proteins KRBP1, PP1c isoforms, and NRL1, targeted by Pi04089 (Wang et al., 2015), Pi04314 (Boevink et al., 2016), and Pi02860 (Yang et al., 2016) effectors, respectively, can be regarded as S factors, in that either their removal reduces *P. infestans* infection, or their overexpression enhances it, or both. The PP1c isoforms can also be regarded as effector helpers (Win et al., 2012), with the genuine targets in the host being the substrates for dephosphorylation by Pi04314-PP1c holoenzymes. NRL1 acts as a negative regulator of immunity in the absence of effector Pi02860, whereas the role of KRBP1 remains unknown. S factors have been shown to be targeted by bacterial T3Es and these recent papers reveal that effectors from biotrophic filamentous eukaryotic pathogens may also target S factors. Future work will reveal how each effector promotes or re-directs the activity of its target, and will identify the cellular substrates in host cells upon which KRBP1, NRL1, and the Pi04314-PP1c holoenzymes act to create a susceptible environment.

The roles of S factors in supporting enhanced susceptibility could be manifested in at least two ways (Figure 1). They could either provide increased nutrients or other metabolites that promote pathogen growth, as typified by the *SWEET* genes (Chen et al., 2010), or they may act directly as endogenous negative regulators of immunity, as shown for MPK4 (Cui et al., 2010). To balance the allocation of energy and resources between growth/development and biotic/abiotic stress responses, plants require an intricate system of cross-talk, comprising positive and negative regulators. These regulators can support growth and antagonize immunity, for example, or vice versa (Figure 1). Recently, antagonism between the growth-promoting brassinosteroid pathway and immune responses has been revealed. A central regulatory component in this cross-talk is the transcription factor BZR1, activated by the BR pathway. BZR1 induces the expression of the basic helix-loop-helix transcription factor HBI1, which is both a positive regulator of BR responses (Bai et al., 2012) and a negative regulator of immunity (Fan et al., 2014). HBI1 is thus a regulator of the trade-off between growth and immunity.

Given that the plant has well-tuned endogenous mechanisms for negatively regulating immunity, it is perhaps unsurprising that biotrophic pathogen effectors have evolved to exploit this (Figure 1). Future work will reveal the extent to which pathogens use the plant's own regulatory systems to antagonize defense, and the roles that effector-targeted S factors play in negatively regulating immunity or otherwise enhancing pathogen growth. A critical step will be in determining whether endogenous negative regulators of immunity play a role also in positively regulating processes that promote growth and development in the plant (Figure 1).

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